**Histological characterization of human photoaged skin by degree of alteration of elastic and collagenic fibers and localization of decorin**

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Chronic sun exposure induces the photoaging of skin including wrinkle formation. A quantitative understanding of the histological alteration of photoaged skin is important for assessing the severity of photoaging. In this study, we made a histological analysis of skin sections from 36 Asian subjects for histological alteration of elastic fibers using Elastica-van Gieson stain and decorin (a marker of the condition of collagen fibers) using immunohistochemistry. Interestingly, the initial alteration of elastic fibers was observed in the deep dermis. Also, in contrast to a decrease in decorin signal intensity with greater alteration of elastic fibers in the reticular dermis, there was an increase in decorin signal intensity just below the basement membrane (the grenz zone). In skin with very severe alteration of elastic fibers, however, decorin localization in the grenz zone was not detected. Based on these histological changes observed in the elastic fibers decorin and collagen, we categorized altered skin tissues into 6 stages of severity (Stage I to VI). The objectivity of our stages was verified by 11 inter-observers, and the degree of agreement was estimated by the weighted kappa statistic. The statistic was 0.889, showing almost perfect agreement between the observers. We also detected a significant positive correlation between photoaging stage and wrinkle score in 26 Caucasian subjects. To conclude, we demonstrated the localization of altered elastic fibers in the early photoaging stage and changes in localization and signal intensity of decorin in the process of photoaging, and provided an objective histological scale correlating with wrinkle scores.

**Development of cell-based *in vitro* models for infrared radiation A (IRA) and thermal exposure**

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Despite of the skin damages caused by UV radiation, IRA and thermal exposure have been considered harmful to skin. In this way, relevant models of single and/or repeated IRA and/or temperature challenge on monolayer skin cells were developed: IR/dT (single and/or repeated IRA challenge; temperature of 42°C); phyIR (single and/or repeated IRA challenge; with temperature maintained at 37°C) and dT (single temperature challenge of 42°C). The models were developed using primary Normal Human Dermal Fibroblasts (NHDF) and primary Normal Human Epidermal Keratinocytes (NHEK). For single IRA challenge of the NHDF, cells were irradiated with 250 J/cm2 for 25 minutes, and for repeated challenges cells were exposed 3 times to 100 J/cm2, for 10 minutes each time. NHEK were exposed to single challenge only (100 or 250 J/cm2 for 13 minutes). Cell viability was tested 24 hours after single or last challenge using MTS assay, and the following biomarkers were assessed: ROS production (DCFDA assay), loss of mitochondrial membrane potential (Δψm; JC-1 dye), MMP-1, MMP-3, MMP-12 and IL-6 gene (qRT-PCR / TaqMan assays) and protein expression (Quantikine ELISA kits), collagen type I (Metra CICP EIA kit) and p38 phosphorylation (Western Blotting). Epigallocatechingallate (EGCG), tetramethylpyrazine (TMP) and quercetin were tested for protective effects. Statistical analysis was performed by ANOVA, using the Dunnet's test ($p < 0,05$; 0,01; 0,001). Some endpoints had significant effects for both cell types, including ROS overproduction, Δψm, MMPs, collagen, IL-6 and p38 phosphorylation. Quercetin 60 μM counteracted the effect of single acute exposure of NHEK. EGCG 10 μM demonstrated specific protection of NHDF on single challenge and quercetin 100 μM on repeated IRA challenge. The results suggest that IRA and thermal exposure can contribute to skin aging.

# 674

**Differential gene expression by simulated solar UV can control epidermal keratinocyte homeostasis**

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Cell response to UV exposure includes exit from the cell cycle into states of quiescence to allow repair, and senescence, differentiation or apoptosis to decrease the number of damaged cells in the stem cell compartment. The choice is dependent upon UV dosage, nutritional status, and capacity for DNA repair; both cell type and strain determine the exact response. In the current study the impact of solar simulated UVR was evaluated using two adult and two neonatal keratinocyte strains (passage 3-5). Dose ranging (0-156 J/cm2) was carried out. At exposures of 10 J/cm2, senescence (b-galactosidase positive cells at 7 days post irradiation) increased ~2-fold and ~4-fold in neonatal and adult keratinocytes, respectively; the percentage of senescent cells in cultures exposed to 10J was 12-16% and 35-90% in neonatal and adult keratinocytes, respectively. Although p16 mRNA levels have been shown to increase with age, UV induced increases in p16 were detected only in the neonatal keratinocytes. Evaluation of the UV response using the GeneSnare Differential Expression Kit and qRT-PCR revealed [1]the predicted increases in the expression of genes within the epidermal differentiation complex (EDC) (e.g., SPRR family members 1B, 2D, 2F, 3, and 4 and S100 family members A7 and P), and [2] increases in the expression of genes involved in regulating epidermal differentiation and cell turnover, but encoded by sequences outside the EDC (e.g., KLK family members 6 and 14). In addition, by 7-day post irradiation decreases in genes expressed within basal keratinocytes (e.g., SPARC, Vimentin, FGFBP1) were observed; decreases in these genes are are consistent with limiting cell turnover and colony expansion. Taken together the data support the notion that epidermal keratinocytes initially suppress the accumulation of damaged cells by promoting differentiation (SPRRs and S100 family) and desquamation (KLK driven), and by reducing rates of epithelial proliferation (reduced SPARC, FGFBP1, Vimentin).

# 675

**UVB irradiation with a monochromatic wavelength of 300-310 nm increases filaggrin expression in a three dimensional human skin model**

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Ultraviolet (UV) phototherapy is a beneficial treatment for many skin disorders, such as psoriasis, atopic eczema, and pruritus. In a murine model, UVB phototherapy improves barrier function by inducing an increase in the expression of terminal differentiation proteins (filaggrin [FLG] and involcrin [IVL]). Recent studies revealed that FLG loss-of-function mutations are associated with the pathogenesis of atopic dermatitis (AD). Histologic evaluation of terminal differentiation proteins, i.e., loricrin (LOR), FLG, and IVL, revealed clear increases in granular layer expression in the keratinocytes of AD patients that received narrowband UVB (NB-UVB) phototherapy. Therefore, we analyzed the action spectrum of UVB for these differentiation molecules. A three-dimensional tissue model of human skin was irradiated with monochromatic UVB (280, 290, 300, 310, 320 nm), NB-UVB, and broadband UVB (BB-UVB). Gene expression was assessed using real-time polymerase chain reaction and protein expression (FLG, Keratin1, Keratin10, LOR, and IVL) was assessed by immunostaining. Following treatment with monochromatic UVB irradiation for 24 to 48 hours, FLG expression was slightly decreased. After 72 hours of UVB irradiation (300 nm, 50 mJ), however, FLG expression reached a maximum (3.1-fold greater than the control). Induction was observed at 300 to 310 nm. Both NB-UVB and BB-UVB increased FLG expression. NB-UVB induced more FLG expression. LOR expression was not changed after monochromatic UVB irradiation, but was increased at 72 and 96 hours after NB-UVB. In contrast, IVL expression remained unchanged. BB-UVB at doses of 10 mJ and 100 mJ increased FLG protein levels after 72 to 96 hours. Monochromatic irradiation at 300 nm and 310 nm also slightly induced FLG after 96 hours. In contrast, the levels of other differentiation proteins were not changed by UVB irradiation. In conclusion, FLG gene expression is increased by monochromatic UVB, NB-UVB, and BB-UVB. Monochromatic UVB at 300 nm leads to increased FLG expression.

# 676

**Toll-like receptor 2 signaling protects against ultraviolet-induced inflammation *in vivo***

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Ultraviolet light (UV) irradiation on skin causes acute inflammatory responses as well as the development of chronic photodamage and skin cancers, and triggers various immune responses. Toll-like receptor (TLR) plays a crucial role linking innate and adaptive immune responses. However, the role of TLR2 signaling in UV-induced inflammation has not been elucidated so far. A single dose of UV irradiation induced the transient up-regulation of TLR2 in mouse and human skin *in vivo*, as well as the interaction of TLR2 with MyD88 or PI3K *in vivo*. Mice with deficient TLR2 (TLR2 KO) showed enhanced UV-induced inflammatory responses, compared with wild-type (WT) counterparts. TLR2 KO mice showed significantly increased epidermal and dermal thickness as well as skin fold thickness, accompanied by an increase of Ki-67 (+) proliferative cells, compared with WT mice. The production of proinflammatory cytokines and chemokines such as IL-1β, IL-6, TNF-α, and CXCL1, and UV-induced apoptosis was significantly augmented in TLR2 KO mice. Of particular interest, UV-irradiated TLR2 KO mice showed significantly less production of anti-inflammatory cytokines IL-10 and SOCS3, and of relevant Foxp3, RANKL, and RALDH2 than WT mice. This aggravation of UV-induced inflammation is at least in part attributed to impaired Akt/GSK3β pathways. Subsequent preconditioning experiments using TLR2 ligands at 24 hr before UV irradiation revealed that, surprisingly, pretreatment of TLR1/2 ligand Pam3CSK4 significantly reduced UV-induced inflammation *in vivo*. Taken together, this study provides an *in vivo* experimental evidence that UV-induced TLR2 signaling protects against acute UV-induced inflammation.

# 677

**Green tea catechins reactivate silenced tumor suppressor genes, p16INK4a and Cip1/p21, in UV-irradiated mouse skin and skin tumors by reducing DNA methylation and increasing histone acetylation**

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Epigenetic alterations can be inherited without changing the DNA sequence and are believed to play a crucial role in ultraviolet (UV) radiation-induced skin cancer. We have previously shown that administration of green tea polyphenols/catechins (GTPs) in the drinking water of mice inhibited UV-induced skin tumor development. It is, however, unclear how GTPs affect UV-induced epigenetic modifications during the prevention of photocarcinogenesis. The present study was designed to investigate whether administration of GTPs would reactivate the expression of tumor suppressor genes in UV-exposed skin and skin tumors. If so, then what is the underlying mechanism? For this purpose, SKH-1 hairless mice were exposed to UVB (180 mJ/cm2) with or without the administration of GTPs (0.2%, w/v) in their drinking water three times per week for 24 weeks. Afterwards, skin tumors and tumor-uninvolved UV-exposed skin samples were collected and subjected to analysis of epigenetic biomarkers using immunohistochemistry, western blotting, enzymatic activity assays and real-time PCR. Our study shows that GTPs decreased the levels of UV-induced: (i) global DNA methylation, (ii) 5-methylcytosine, (iii) DNA methyltransferase (Dnmt) activity, (iv) mRNA and protein levels of Dnmts. GTPs inhibited UVB-induced histone deacetylation (HDAC) activity and increased levels of histone acetylation on histones H3 and H4. GTPs resulted in reactivation of the mRNA and proteins of UVB-induced silenced tumor suppressor genes, p16INK4a and Cip1/p21. Identical chemopreventive effects of GTPs were found when UVB-induced skin tumors from mice treated with or without GTPs were analyzed for tumor suppressor genes, the levels of Dnmts, and HDAC activity, etc. Together, these data provide insight into the epigenetic mechanisms of GTPs against UV radiation effects which may contribute to the prevention of photocarcinogenesis and may have implications for epigenetic therapy.

# 678

**Proanthocyanidins inhibit UV-induced immunosuppression through DNA repair-dependent functional activation of dendritic cells**

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Dietary intake of grape seed proanthocyanidins (GSPs) inhibits ultraviolet (UV) radiation-induced immunosuppression in mice. However, it is not understood whether GSPs target dendritic cells (DC) while exerting its immunomodulatory effects. To better understand this concept, we first determined the DNA repair ability of GSPs in UV-exposed DC. For this purpose, CD11c+ bone marrow-derived DC were prepared from xeroderma pigmentosum complementation group A-knockout (XPA-KO) mice as well as their wild-types (WT). CD11c+ cells were exposed to UV radiation with or without pretreatment with GSPs. Treatment with GSPs enhanced repair of UV-induced DNA damage when determined in terms of the number of cyclobutane pyrimidine dimer (CPD)+ cells in CD11c+ cells from WT mice, but this DNA repair effect of GSPs was not seen in the CD11c+ cells from XPA-KO mice. We then determined *in vivo* effects of dietary GSPs (0.5%, w/w) on secretion of cytokines by CD11c+ cells that were isolated from lymph nodes of mice (WT and XPA-KO) exposed to UV. Our results showed that CD11c+ cells isolated from mice that received dietary GSPs secreted higher levels of IL-12 and IFNγ and lower levels of IL-10 compared to the levels of these cytokines in CD11c+ cells obtained from control (non-GSPs) mice. However, this effect of GSPs was not found in CD11c+ cells from XPA-KO mice. Finally, we used an adoptive transfer approach, wherein draining lymph nodes were harvested from mice exposed to UV with and without dietary GSPs treatment and sensitized with DNFB. CD11c+ cells were isolated and transferred into naïve mice that were subsequently challenged with DNFB on the ear skin. Naïve recipients that received CD11c+ cells from WT, GSPs-treated, UV-irradiated donors exhibited full contact hypersensitivity (CHS) response, whereas no significant CHS was observed in naïve XPA-KO mice. This data suggests that dietary GSPs inhibit UV-induced immunosuppression by promoting the functional ability of DC through enhanced repair of UV-induced DNA damage.

# 679

**The acute response to UVB in human skin includes infiltration of multiple dendritic cell subtypes and activation of innate defense molecules**

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The acute response of human skin to ultraviolet B (UVB) radiation has not been fully characterized. We sought to define the acute response of human skin to narrowband UVB (312 nm peak) 24 hours following irradiation using transcriptional profiling, immunohistochemistry, and immunofluorescence. As expected, Langerhans cells and T cells were decreased, and there were increased myeloid inflammatory CD11c+BDCA1- dendritic cells in irradiated skin. In addition, there were increased CD11c+BDCA3+ dendritic cells, a subtype that is thought to cross-present antigens to CD8 T cells and has not been previously characterized as part of the response to UVB. There was increased protein expression of inflammatory markers TNF and TRAIL in irradiated skin, which co-localized with dendritic cell marker CD11c. There was absence of co-localization of CD11c and DC-LAMP, suggesting that these cells are immature dendritic cells. In the transcriptome, there was upregulation of dendritic cell-attracting chemokines (CCL20, CCL2), both inflammatory and anti-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10), and antimicrobial peptides (S100A7, S100A12, human beta-defensin 2, and elafin), which were confirmed by RT-PCR or immunohistochemistry staining. Analysis of microarray results with Ingenuity Pathway Analysis, which included 1,522 unique differentially expressed genes, demonstrated activation of multiple innate and early adaptive immune pathways. These results show that the acute response of human skin to erythrogenic doses of UVB includes activation of innate defense mechanisms as well as infiltration of multiple subtypes of inflammatory dendritic cells that could serve to link innate and adaptive immunity.

# 680

**AMPK activation prevents UVB-induced skin tumorigenesis through promoting DNA repair and growth control**

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Non-melanoma skin cancer is the most common cancer in the U.S., while DNA-damaging UVB radiation from the sun remains the major environmental risk factor. AICAR (5-aminoimidazole-4-carboxamide ribonucleoside) and metformin (N',N'-dimethylbiguanide), the most widely used anti-diabetic drug, are activators for the energy-sensing enzyme 5'-AMP-activated protein kinase (AMPK). Here we show that AMPK activation is reduced in human and mouse squamous cell carcinoma as compared with normal skin, and by UVB irradiation, suggesting that AMPK is a tumor suppressor. Topical AICAR and metformin not only delayed onset of UVB-induced skin tumorigenesis but also reduced tumor multiplicity. At the cellular level, AICAR and metformin increased the expression of the DNA repair protein xeroderma pigmentosum C (XPC) and UVB-induced DNA repair, and inhibited ERK activation and cell proliferation in keratinocytes and mouse skin, while AMPK deletion reduced XPC expression and DNA repair, and increased ERK activation and cell proliferation. AICAR and metformin enhanced repair of UVB-induced DNA damage through activating the AMPK pathway. In contrast, they inhibited cell proliferation through an AMPK-independent pathway. Furthermore, in UVB-damaged tumor-bearing mice, both topical and systemic metformin prevented the formation of new tumors and suppressed growth of established tumors. Our findings not only suggest that AMPK is a tumor suppressor in the skin by promoting DNA repair and controlling cell proliferation, but also demonstrate that the AMPK activators AICAR and, in particular, the anti-diabetic drug metformin, may be promising agents for skin cancer chemoprevention and intervention in high risk individuals with skin cancer history.

# 681

**Diclofenac decreases tumor burden in male and female mice in two models of UVB-induced SCC**

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While the risk of developing cutaneous SCC is 3 times greater in males than females, the mechanisms that contribute to this disparity are unclear. Our previous studies showed that after equivalent, chronic UVB exposure, male mice had greater tumor multiplicity, burden, and grade compared to female mice. Additionally, our acute UVB studies revealed decreased total antioxidant capacity and cutaneous inflammation in male mice, suggesting that topical antioxidant or anti-inflammatory agents might decrease tumor burden following chronic UVB exposure. In the current study we examined the effects of antioxidant/anti-inflammatory agents on UVB carcinogenesis using two UVB protocols modeling a compliant or a non-compliant patient. In the compliant model, male and female Skh-1 mice were exposed to UVB for 10 wks then treated with a topical antioxidant (catalase, vitamin E, or CE Ferulic) or anti-inflammatory (Diclofenac) for 15 weeks without additional UVB exposure. While only male mice exhibited a moderate decrease in tumor burden with vitamin E and catalase, CE Ferulic and Diclofenac decreased tumor burden by 46% and 73% respectively, in both sexes. In the non-compliant model, mice were exposed to UVB for 25 weeks, with topical treatments applied after each UVB exposure starting at week 11. In this model, only topical Diclofenac treatment decreased tumor burden in either male or female mice. Our data illustrate a clear separation in tumor burden between the 10 wk and 25 wk UVB studies, with the lowest burden of the 25 wk study being equivalent to that of the highest tumor burden of the 10 week study. Importantly, our findings demonstrate the benefits of topical antioxidant treatments in our compliant model compared to limited effects in the non-compliant model. Furthermore, the current studies underscore the importance of inflammation in UVB-induced tumor development regardless of the length of UVB exposure. These results demonstrate both the dangerous effects of cumulative UVB exposure and the importance of limiting UVB exposure to achieve therapeutic benefits.

# 682

**Induction of immature pigment cell development is significantly affected by the irradiance of UVB therapy**

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Wavelengths and fluences administered have been considered as the crucial parameters responsible for the efficacy of phototherapy. Little attention has been paid to the effect of irradiance. Narrow band ultraviolet B (NBUVB; 311nm) and excimer (308nm) lights emit radiation at similar wavelengths with excimer having much higher irradiance. Previous reports have shown that excimer light is more effective than NBUVB for treating vitiligo. In this study, we demonstrated the superior efficacy of excimer light than NBUVB for treating vitiligo in terms of more rapid initial repigmentation and better repigmentation profiles after 3 months. Using primitive mouse-derived melanoblast cells to recapitulate vitiligo repigmentation *in vitro*, we demonstrated that at equivalent fluence, excimer light induced less viability reduction as compared to NBUVB ($p<0.05$). In terms of pro-differentiation effect on melanoblasts, we showed that excimer light 1). stimulates more prominent nuclear translocation of epidermal growth factor receptor (EGFR), 2). induces physical binding between EGFR and tyrosinase promoter sequence, and 3). is capable of initiating tyrosinase transcription and translation while equivalent fluence delivered by NBUVB failed to do so. Reduction of excimer irradiance by filter abrogated 1). nuclear translocation of EGFR, 2). binding between EGFR and tyrosinase promoter sequence, and 3). transcription of tyrosinase, even if equivalent fluence was delivered. In conclusion, we demonstrated that irradiance is a crucial parameter for successful phototherapy for vitiligo. As phototherapy is frequently used for medical and aesthetic purposes currently, a new paradigm should be established incorporating irradiance as a crucial parameter for phototherapeutic treatment.

# 683

**UVR regulates local cortisol production and GR expression in a wavelength dependent manner**

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11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1; HSD11B1, gene) reduces cortisone to cortisol (COR), which activates glucocorticoid (GC) receptor (GR); while 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2; HSD11B2, gene) oxidizes COR to cortisone and prevents illicit activation of the mineralocorticoid receptor. These two enzymes, together with GR, play a key role in tissue-specific regulation of GC action in the skin. Since ultraviolet (UV) radiation is a stressor for the skin inducing the local and systemic immunosuppression, we investigated the expression of the 11β-HSD1/2 as well as GR at the gene and protein levels after irradiation of UVA, UVB and UVC in human skin explants and/or co-cultured keratinocytes/melanocytes. In cell culture, UVA stimulated HSD11B2/11β-HSD2, having no significant effects on 11β-HSD1 protein levels and COR production. The GR mRNA was stimulated by UVA and inhibited by UVB in a dose dependent manner, with significant changes in protein expression seen only for UVB and UVC. The highly energetic UVB and UVC, but not UVA, enhanced the HSD11B1/11β-HSD1 expression with attendant COR production, and decrease in GR expression. The *in situ* immunofluorescence (IF) showed the cytoplasmic localization of 11β-HSD1/2 antigens with changes in immunofluorescent intensity in the basal and upper epidermal layers after respective UV doses. We propose that these differential effects are both to protect the epidermal barrier build by keratinocytes against disruption by elevated cortisol (inhibition of GR in epidermis), and to attenuate autoimmune responses triggered by UVB/C induced damage trough cortisol action on immune cells (stimulation of pathways increasing cortisol).

# 684

**Cartilage oligomeric matrix protein increases in photodamaged skin**

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The degeneration of the dermal extracellular matrix is induced by chronic sun exposure, which is thought to be associated with the formation of wrinkles, laxity, pigmentation, and the fragility of aged skin. However, the mechanisms leading to the degeneration of connective tissue in photodamaged skin are not clear. To investigate the gene expression profile of photodamaged skin, we performed a microarray analysis using RNAs extracted from preauricular skin (photodamaged area) and postauricular skin (photo-protected area) from the same subject. Among 4 up-regulated genes, we focused on cartilage oligomeric matrix protein (COMP), an extracellular matrix protein and the fifth member of the thrombospondin family. *In situ* hybridization revealed that COMP mRNA positive cells increased with the progress of solar elastosis in the pre/postauricular skin dermis. In addition, immunohistochemical staining showed that the COMP protein also increased with dermal elastosis in pre/postauricular skin dermis. When recombinant COMP was incubated with normal human dermal fibroblast, it promoted collagen fibrillogenesis and suppressed the deposition of elastin around fibroblasts. These results suggest that COMP is involved in the organization of the extracellular matrix in photodamaged skin.

# 685

**DNA lesions and apoptosis induction in a new human epidermis model exposed to solar radiation**

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Ultraviolet radiation present in sunlight is a major environmental human carcinogen that also contributes to photoaging process. Today, tissue engineering allows the *in vitro* reconstruction of skin substitutes for studying photobiology and photoprotection in the cosmetic industry. However, the deleterious effects of solar-simulated radiation (SSR) are not fully understood in these alternative skin models. In this study, the phototoxicity of a single acute SSR dose of 16.5 J/cm2 was evaluated in a newly engineered human epidermis. SSR induced significant tissue damage 24h after irradiation as assessed by tetrazolium salt reduction (MTT) assay and lactate dehydrogenase leakage in the culture medium. Loss of skin viability was associated to the formation of DNA lesions, thymine cyclobutane dimers and DNA fragmentation (CPD immunohistochemistry and ELISA, TUNEL technique). SSR was also correlated to apoptosis induction since sunburn cells were clearly detected by histology in the epidermis whereas the caspase-3 protease was strongly activated under SSR exposure as demonstrated by both enzymatic assay and immunostaining. Interestingly, the DNA alterations coincided with the activation of the caspase-3 in the basal keratinocytes. None of the above cellular responses was observed in non-irradiated epidermis, and the topical application of a broad-spectrum UVB + A sunscreen (SPF30, 2 mg/cm2, Laboratoires Avène) afforded an efficient photoprotection. Thus, the new human epidermis model may be a good surrogate to human skin. Finally, it may be useful to assess SSR-induced photodamage and to evaluate the efficacy of sunscreen formulations.

# 686

**The gene expression profile of betaine- and UVB-treated organotypic keratinocyte cultures**

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Ultraviolet radiation is known to inflict epidermal DNA damage through photon absorption by DNA or generation of reactive oxygen species (ROS). Betaine, also known as trimethylglycine, is a stable, non-toxic, and highly water-soluble molecule. Betaine is widely used in cosmetic products because of its moisturizing and protective properties, however, there are few studies addressing these effects in the skin. The purpose of this study was to investigate whether betaine tends to have a protective role against UVB. In this project organotypic rat keratinocyte cultures were exposed to a single dose of UVB (30 mJ/cm$^2$), 10 mM betaine or their combination. Global gene expression was determined using Illumina RatRef-12 expression BeadChip. Based on the microarray data, 15 genes were chosen and their expression levels were confirmed using qPCR. In addition, the expression of keratin 2, keratin 10 and filaggrin were studied using western blotting and immunohistochemistry. Expression of 627 genes was changed significantly after UVB-exposure whereas betaine alone changed the expression of 89 genes. UVB upregulated 362 genes, 222 of which were also upregulated after betaine pretreatment. This signifies that 140 genes were unique to the UVB-treatment, or betaine pretreatment prevented their induction. Biological processes altered by UVB were related to DNA replication, cell cycle and DNA repair whereas betaine treatment regulated TGF-β signaling, response to cytokines and certain metabolic processes. Betaine significantly influenced the expression of keratin 2, but not the expression of other keratins or epidermal differentiation markers. In conclusion, betaine has some specific, unique effects on keratinocyte gene expression; the biological relevance of these changes awaits confirmation.

# 687

**A method for analyzing the protective effect against UVB stress in a human epidermal 3D culture model**

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Acute exposure to UVB irradiation provokes sunburn, inflammation and immunosuppression. Although sunscreen is widely used for prevention of sunburn, there is no medical treatment to relieve sunburn. Most molecular data are obtained from monolayer cell culture, which does not reproduce the condition of integral skin. A human epidermal 3D culture model, EPI-201, which was obtained from MatTek was used to study UVB-induced effects and analyze a panel of biomarkers. The EPI-201 model was irradiated with UVB at single dose of 600 mJ/cm2 using FL20S-E lamps and cultured for up to 24 h after irradiationIL-1 alpha and COX-2 gene expression was induced about two-fold in the early phase., heme oxygenase-1 (HO-1) and elafin were induced about ten-fold and two-fold, respectively, in the late phase. These data indicated that UV irradiation first elicited gene expression associated with an inflammatory response followed by an oxidative stress response later. Topical application of niacinamide improves symptoms of photoaging and remedies immunosuppression. However, it is unclear whether topical niacinamide is effective on sunburn inflammation. The effects of niacinamide were examined by adding an aqueous solution to the stratum corneum immediately after UVB irradiation. Strikingly, niacinamide decreased UVB stress, such as induction of IL-1 alpha or HO-1 gene expression. In addition, niacinamide inhibited poly ADP-ribose polymerase-1 (PARP-1) activity. PARP-1 inhibitors show a therapeutic effect on various inflammatory disease models, therefore, it could be a possible mechanism for the anti-inflammatory effect. This study established an *in vitro* model for sunburn to investigate both inflammation and oxidative stress. Furthermore, this study demonstrated the alleviating effect of niacinamide on sunburn inflammation.

# 688

**Photo(chemo)therapy reduces circulating Th17 cells and restores dysfunctional regulatory T cells in psoriasis**

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Photo(chemo)therapy is widely used to treat psoriasis, the pathogenesis of which might be caused by an imbalance of Th17 cells and regulatory T cells (Treg). In the present study, we evaluated the effects of photo(chemo)therapy on the Th17/Treg balance in peripheral blood obtained from psoriasis patients treated with bath-psoralen ultraviolet A (n=50) or narrowband ultraviolet B (n=18), and healthy volunteers (n=26). Th17 and Treg (CD4+CD25+Foxp3+) cells were analyzed by FACS. CD4+T cells from patients with increased Th17 (Th17/CD4 >3.0 [mean+SD of healthy volunteers]) were analyzed before and after photo(chemo)therapy. Photo(chemo)therapy significantly reduced Th17 cells from 5.7±3.1% to 3.0±2.9%. In contrast, photo(chemo)therapy significantly increased Treg from 2.7±0.7% to 3.4±1.3% in patients with decreased Treg (Treg/CD4 <3.8 [mean of healthy volunteers]). Next, we evaluated whether Treg function is impaired in psoriasis. CD4+CD25+Treg isolated using MACS beads and CD4+CD25-responder T cells labeled with CFSE fluorescence were co-cultured with anti-CD3, CD28 beads for 4 days. The Treg Functional Ratio (%), defined as %Suppression of responder cell proliferation by Treg, was significantly lower in patients (72.1±24.8% n=15) than in controls (94.4±4.3% n=9, p=0.015). Moreover, the Treg Functional Ratio was significantly increased by bath-psoralen + ultraviolet A therapy, restoring Treg function to almost normal levels (from 67.7±27.1 to 88.9 ±5.5% n=11, p=0.030). In addition, the Treg Functional Ratio was inversely correlated with the Psoriasis Area and Severity Index score (r= -0.407 p=0.084), and the product of the Treg Functional Ratio and the percentage of CD25+FoxP3+/CD25+ cells was inversely correlated with the Th22 level (r= -0.380 p=0.108). These findings confirm that Treg are dysfunctional in psoriasis patients, and that photo(chemo)therapy restores Treg function in most patients. Photo(chemo)therapy resolved the Th17 and Treg imbalance in patients with psoriasis.

# 689

**Photodynamic therapy with pc 4 induces cell death of *trichophyton rubrum***

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Onychomycosis is the most prevalent nail disease in adults and *Trichophyton rubrum* is the most common pathogen. Furthermore, *T. rubrum* is responsible for the most common causes of dermataphytosis. Treatment is limited since topical antifungals are only partially effective and systemic agents, such as terbinafine, have serious adverse effects including liver damage. Photodynamic therapy (PDT) is a non-invasive treatment that uses a photosensitizing drug and light, which, in the presence of oxygen in the tissue results in the destruction of pathologic cells or microbes. We have previously demonstrated that PDT using the silicon phthalocyanine Pc 4 can induce apoptotic cell death in *C. albicans in vitro* . In this study, we demonstrate the *in vitro* toxicity of Pc 4-PDT in *T. rubrum*. Confocal image analysis confirmed that Pc 4 permeates the cell wall and localizes to cytosolic organelles. Increased fungal killing was observed with increasing doses of Pc 4 from 0 to 2 μM followed by light at 1.0 J/cm$^2$. This was confirmed using a clonogenic assay. Similarly, the XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide) assay showed that Pc 4-PDT impaired fungal metabolic activity in a Pc 4 dose-dependent manner. Of interest is that these results were obtained in both terbinafine-sensitive (23103) and terbinafine-resistant (MRL666) strains, indicating the potential of Pc 4-PDT as an effective agent against *T. rubrum*. Given that Pc 4-PDT has already demonstrated an excellent safety profile in Phase 1 clinical trials for skin cancer and psoriasis, we aim to initiate a pilot clinical trial to determine the efficacy of Pc 4-PDT *in vivo*.

# 690

**20-Hydroxylvitamin D3 possesses high efficacy against proliferation of cancer cells while being non-toxic**

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Recently 20-hydroxyvitamin D3 (20(OH)D3) has been defined as a novel natural product with antiproliferative and pro-differentiation properties towards human keratinocytes. To define its potential utility as a tumorostatic agent we assessed its ability to inhibit cancer cell growth *in vitro*, and tested *in vivo* toxicity. The anti-tumor activity of 20(OH)D3 was tested against breast and liver cancer cell lines using colony formation assays. We also tested the effect of 20(OH)D3 on NF-κB protein expression in keratinocytes and compared it with 1α,25-dihydroxyvitamin D3 (1α,25(OH)2D3).To assess *in vivo* toxicity, mice were injected with 20(OH)D3 intraperitoneally (i.p.) each day for 3 weeks. Blood and organ samples were collected for clinical pathology, chemistry and histology analyses. 20(OH)D3 displays similar tumorostatic activity against MDA-MB-453 and MCF7 breast carcinomas, and HepG2 hepatocarcinoma, and inhibits NF-κB protein expression, in a dose-dependent manner. This compound is not hypercalcemic, does not cause detectable toxicities in liver, kidney, or on blood chemistry in mice at a dose as high as 30 μg/kg. In contrast, both 25(OH)D3 and 1,25(OH)2D3 caused severe hypercalcemia at a dose of 2 μg/kg. 20(OH)D3 possesses high efficacy for inhibiting cancer cell proliferation *in vitro* and is non-toxic *in vivo*, supporting its further development as a potential anti-cancer therapeutic agent.

# 691

**Cosmetic formulations that contain botanicals with natural antioxidant activities quench free radicals as measured by the Briggs-Rauscher reaction method**

TC Florence, D Gan, G Kalahasti and MD Hines *Skin Care Research, Mary Kay, Inc., Dallas, TX*

Cosmetic topical formulations frequently promote antioxidant protection from free radicals in the environment. Although the individual ingredients in these formulations may quench free radicals *in vitro*, the formulations are not normally tested for antioxidant capacity. We compared the *in vitro* antioxidant capacities of individual ingredients measured by the TEAC assay in various commercial formulations to the antioxidant capacities of the full formulations measured by the Briggs-Rauscher method. We found that formulations containing botanicals with demonstrated antioxidant activity in the TEAC assay showed comparable antioxidant capacity in the Briggs-Rauscher method. Interestingly, a formulation containing only tocopheryl acetate did not provide antioxidant capacity as measured by the Briggs-Rauscher reaction. These data demonstrate that the Briggs-Rauscher method may serve as a valuable tool for measuring true antioxidant capability in topical cosmetic formulations.

# 692

**A cosmetic formulation containing ingredients that stimulate the production of matrix proteins *in vitro* improves skin texture, laxity, sagging, and overall appearance *in vivo***

TC Florence, D Gan, G Kalahasti and MD Hines *Skin Care Research, Mary Kay, Inc., Dallas, TX*

Ascorbic acid (Vitamin C) is widely known to improve the appearance of photodamaged skin. However, inclusion of ascorbic acid in topical cosmetic formulations is challenging as it is prone to rapid oxidation. In this study, we evaluated five botanical extracts known to naturally contain high levels of ascorbic acid. Utilizing *in vitro* testing methods, we found these extracts inhibited matrix metalloproteinase activity. In addition, these extracts stimulated synthesis of collagen, fibronectin, and laminin in normal human fibroblasts. Application of topical cosmetic serum containing these extracts to three dimensional skin constructs stimulated the production of dermal matrix proteins. We conducted a clinical study and subjects showed significant improvements in skin texture, laxity, sagging, and overall appearance.

# 693

**Vitamin D3 activates DNA repair pathways in UV-irradiated human melanocytes**

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We investigated the effects of 1,25(OH)2 vitamin D3 (vit D3), known to be synthesized by epidermal keratinocytes in response to UV exposure, on the UV-induced DNA damage in cultured human melanocytes (MC). By Western blotting, we found that MC express functional vitamin D receptor (VDR), which was up regulated by treatment with vit D3 or α-melancortin (MSH). Treatment with100 nM vit D3 for 4 days prior to, and 48 h post irradiation with 90 mJ/cm2 UV significantly enhanced the repair of cyclobutane pyrimidine dimers (CPD), and reduced UV-induced apoptosis. We have previously reported that the melanocortin 1 receptor (MC1R) genotype is a determinant of the DNA repair capacity of MC. The effects of vit D3 on CPD repair were evident even in MC expressing non functional MC1R, suggesting that vit D3 can compensate partially for loss of function of MC1R. An early response to UV is activation of ATR, which then activates ATM. These kinases phosphorylate E2F1, which is essential for global genome repair since it allows for co-localization of XPC, XPA and p62 at DNA damage sites. Treatment with vit D3 for 4 or 8 h increased the levels of E2F1. Vitamin D3 also increased the levels of XPC, which is involved in the recognition step in nucleotide excision repair (NER) pathway, within 60 min, and for at least 4 hours. The levels of XPA were also transiently increased within 30 min of vit D3 treatment. The antioxidant enzymes OGG1 and APE1, known to participate in base excision repair (BER), were increased in expression 24 and 4 hours after vit D3 treatment, respectively. Translesion synthesis, the pathway that repairs residual DNA damage after NER and BER, was also activated by vit D3, as evidenced by increased levels of Pol η and Rad 18 within 30 and 60 minutes of treatment. In conclusion, vit D3 activates NER, BER and TLS in melanocytes, thus it is expected to reduce UV-induced mutagenesis and melanoma formation. We propose that vit D3 might be efficacious for melanoma prevention.

# 694

**Proteomic identification of biomarkers of photoprotection in *in-vivo* human skin**

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The aim of our study was to use a proteomics-based analysis to determine protein biomarkers of sun protection on human skin *in vivo* after simulated solar radiation (SSR). Four 2.5cm$^2$ areas of full-thickness suction blister epidermis were collected from twelve healthy participants (age: 19-53; FST I-IV). The areas were treated as follows: 1) unirradiated control, 2) SSR-irradiated, 3) sunprotection control, and 4) sunprotection plus SSR-irradiated. The epidermal cell proteome was separated using a 1D gradient SDS PAGE-fractionation approach followed by a label free quantitation of differentially expressed proteins using chromatographic peak volume compilations from the Rosetta Elucidator data analysis platform. A total of 1930 proteins were successfully quantified based on 8378 peptides. Using a 2-way factorial statistical analysis, a total of 365 proteins were found to be differentially regulated and having an interaction effect due to the sun protection product application on unirradiated control vs SSR-irradiated patient samples. This approach identified a number of abundant epidermal proteins that are significantly modulated by SSR and normalized by photoprotection (i.e. Keratin 16, STAT3 and FRYL are among the proteins exhibiting such behavior). Protein-protein interaction analysis of the protein expression data using the Ingenuity Pathway Analysis (IPA) software reveals the suppression of Caspase and NFkB pathways suggesting an elevated anti-apoptotic and anti-inflammatory cellular response due to the protective sunscreen effect following the acute UV insult. The gel-based fractionation approach was very powerful in mining the epidermal tissue proteome in spite of the dominating keratin levels. Novel protein marker panels will allow alternative approaches to evaluating sun protection efficacy.

# 695

**Toll-like receptor-4 augments ultraviolet radiation induced cutaneous tumor development by DNA damage mechanism**

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Ultraviolet (UV) B radiation (290-320 nm) induced DNA damage is an important trigger for suppression of immune responses and for the initiation of non-melanoma skin cancers. UVB causes DNA damage, predominantly in the form of cyclobutane pyrimidine dimers (CPD). Reactive oxygen species (ROS), which are generated endogenously by cellular oxygen metabolism or exogenously by UV, also produce various types of DNA damage. 8-Oxo-2′-deoxyguanosine (8-Oxo-dG) is one type of oxidative DNA damage that can result in stable mutations. Toll-like receptor 4 (TLR4), a component of innate immunity, has been shown to play an important role in cancer. Previous studies from our laboratory indicate that TLR4 deficient mice developed significantly fewer CPD ($p<0.05$) in their skin upon UVB exposure. Our recent experiments indicate that when mice are exposed to single dose of UVB (200 mJ/cm2), UVB-induced DNA damage mediated by ROS is greatly reduced in TLR4 deficient mice, indicated by significantly fewer 8-Oxo-dG lesions ($p<0.05$) in the skin of these mice. We also found that when mice were exposed to multiple doses of UVB radiation (200 mJ/cm2), cutaneous carcinogenesis was retarded in terms of tumor incidence, and tumor latency, in mice deficient in TLR4 compared to TLR4 competent mice, whereas significantly greater ($p<0.05$) numbers of tumors occurred in TLR4 competent mice. Further, we found that CD4+CD25+ regulatory T-cells from TLR4 competent mice produced more IL-10 ($p<0.05$) than regulatory T-cells from TLR4 deficient mice. There was a significant increase ($p<0.05$) in Foxp3 expression in skin of TLR4 competent mice than TLR4 deficient mice. Together, our data indicate that TLR4 mediated UVB induced DNA damage in the form of CPD and 8-oxo-dG lesions maybe a molecular trigger for development of UVB induced skin cancers. Thus, strategies to inhibit TLR4 may allow us to develop immunopreventive and immunotherapeutic approaches for management of UVB induced cutaneous DNA damage and skin cancer.

# 696

**LED generated low level light therapy inhibits human skin fibroblast proliferation while maintaining cellular viability**

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Light emitting diodes (LED) have clinical advantages compared to other light sources, and therefore their use is increasing in clinical dermatology for clinical applications such as chronic venous ulcers, acne and actinic keratosis. However, the effects of LED produced low level light therapy (LLLT) on dermal fibroblast biology is not well understood. To evaluate these effects, cultured human skin fibroblasts were irradiated with a commercially available, handheld, dual wavelength LED device (red light, 633nm and near infrared light, 830nm) at different fluences (80 J/cm$^2$ to 320 J/cm$^2$) and their proliferation and viability were studied using a trypan blue assay. Fluences of 160 J/cm$^2$ and 320 J/cm$^2$ of red light and 80 J/cm$^2$, 160 J/cm$^2$ and 320 J/cm$^2$ of near infrared light decreased fibroblast proliferation ($p < 0.01$) with no significant effect on cell viability. We propose that this novel property of LED generated low level light therapy may hold clinical promise for the treatment of hyperprolific skin disorders such as scars, hypertrophic scars and keloids, since it decreases fibroblast proliferative ability yet maintains viability.

# 697

**Human dermal fibroblasts protect against UVB-induced keratinocyte damage in a human skin equivalent model**

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Epidermal-dermal crosstalk is critical for a variety of processes including wound-healing and the maintenance of the epidermis. The aim of this study was to investigate how fibroblast-keratinocyte interactions affect keratinocyte responses to ultraviolet B (UVB) radiation. A human skin equivalent model consisting of primary human dermal fibroblasts and keratinocytes on a processed dermal scaffold was used to study UVB-induced damage, death and repair in the epidermis. The presence of fibroblasts significantly decreased UVB-induced apoptosis ($p<0.05$) and increased the rate of cyclopyrimidine dimer repair in keratinocytes ($p<0.05$). Additionally, corresponding to the enhanced repair, fibroblasts also modulated keratinocyte cell cycle arrest at the G0/G1 phase. The regeneration of the epidermis in the skin equivalent composites was also accelerated with the presence of dermal fibroblasts. These results indicate that fibroblasts play a critical role in the acute responses of keratinocytes to UVB, the dysregulation of which may be implicated in the development of skin cancers. This study also highlights the use of a skin equivalent model as a tool for studying the responses of skin to ultraviolet radiation.

# 698

**Regulatory T cells induced by the AhR ligand nonylphenol express GARP**

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Ultraviolet radiation (UVR) suppresses the immune system via induction of regulatory T cells (Treg). The arylhydrocarbon receptor (AhR), a cytoplasmic detoxification receptor, can be activated by UVR in a ligand-independent fashion. The AhR seems to be involved in UVR-induced immunosuppression since the induction of Treg by UVR is prevented by AhR antagonists. Hence, we postulated that in turn activation of the AhR by ligands should exert immunosuppression. Injection of the AhR ligand nonylphenol (NP) into mice prevented the induction of sensitization against dinitrofluorobencene (DNFB). In addition, Treg were induced as shown by adoptive transfer. NP-induced Treg act antigen-specifically since Treg obtained from DNFB- and NP-treated donors only blocked the sensitization against DNFB but not against oxazolone in the recipients. GARP (glycoprotein A repetitions predominant) is a protein specifically expressed in Treg upon activation of the T cell receptor. This is functionally relevant since silencing of GARP in Foxp3-expressing Treg inhibits their suppressive activity. To determine whether NP-induced Treg express GARP, mice were injected i.p. for 4 days with NP. 24 h after the last injection mice were sensitized against DNFB. 5 days later lymph nodes were obtained for i.v. injection into naïve mice. Before transfer, cells were depleted from GARP-expressing cells by magnetobead separation using a monoclonal anti-mouse-GARP antibody. 24 h after injection animals were sensitized with DNFB and after 5 days ear challenge was performed. The contact hypersensitivity (CHS) reaction in recipient mice injected with unfractionated cells from NP-treated donors was remarkably suppressed. In contrast, transfer of GARP-depleted cells did not inhibit sensitization. In turn, injection of GARP-enriched cells resulted in a pronounced suppression of CHS. This suggests that the AhR ligand NP induces Treg which express GARP. It is currently under investigation by which mechanisms GARP mediates the suppressive activity of Treg.

# 699

**TRPV1 inhibitory peptide prevents UV-induced skin responses**

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Transient receptor potential vanilloid 1 (TRPV1) channel can be activated by vanilloids, exposure to ultraviolet (UV) light, heat, or protons, and conditions that occur during tissue injury. In the present study, we designed new TRPV1 inhibitory peptide mimicking the phosphorylated site by both protein kinase C (PKC) and $Ca^{2+}$/calmodulin-dependent protein kinase (CAMK) in TRPV1 (a.a 701-709 : QRAITILDT), and investigated whether this TRPV1 inhibitory peptide can reduce UV-induced responses. Treatment with this TRPV1 inhibitory peptide prevented UV-induced mRNA and protein expression of MMP-1 and pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α in HaCaT cells. Treatment with this peptide also inhibited UV-induced calcium influx in HaCaT cells. TRPV1 inhibitory peptide inhibited UV-induced skin thickening, as measured by a caliper, or in hematoxylin and eosin (H&E) stained sections, and also prevented UV-induced apoptosis measured by TUNEL staining in the skin of hairless mice. UV-induced mRNA and protein expression of MMP-13 and MMP-9 was significantly reduced by the peptide, while UV-induced procollagen decrease was recovered by it. In conclusion, our results suggest that a novel TRPV1 blocker, our TRPV1 inhibitory peptide, could prevent UV-induced skin responses, and provide new insight into development of effective therapeutic methods for photoaging.

# 700

**Expression of catalytically active Matrix Metalloproteinase-1 in transgenic mice induces dermal extracellular matrix degradation and fibroblast alterations that resemble photoaged human skin**

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Matrix metalloproteinases 1 (MMP-1) initiates cleavage of type I collagen, which underlies deleterious alterations in the dermis in aged and photoaged human skin. To gain insight into the role of MMP-1 in skin aging, we generated transgenic mice expressing catalytically-active mutant form of MMP-1 (MMP-1 V94G). Fragmented collagen, quantified by HPLC, was 2-fold greater in MMP-1 V94G transgenic mice, compared to non-transgenic littermates ($n=10$, $p<0.05$). Collagen fibrils, visualized by Masson's trichrome stain, appeared disorganized and fragmented in the papillary dermis of MMP-1 V94G mice. Fibroblast morphology and interactions of fibroblasts with collagen fibrils was assessed by electron microscopy and immunohistology. Fibroblasts adjacent to fragmented collagen fibers displayed a collapsed rounded morphology with reduced cytoplasmic area. These collapsed fibroblasts lacked intimate contact with intact collagen fibrils, as observed in non-transgenic littermates. Immunohistology of integrin β-1 (primary collagen receptor) revealed disruption of focal contacts in MMP-1 V94G transgenic mice. Furthermore, protein carbonyls, reflecting oxidative injury, were significantly increased (~17%) in MMP-1 transgenic mice, compared to non-transgenic littermates ($N=10$, $p<0.05$). Oxidative injury correlated with decreased expression of anti-oxidant enzyme SOD-1 (reduced 20%, $N=10$, $p<0.05$). Expression of MMP-1 in transgenic mice recapitulates many of the prominent features of aged human skin, including increased collagen fragmentation, disorganized collagen fibrils, collapsed fibroblast morphology and increased oxidative stress. Therefore, taken together our results support the concepts that elevated expression of MMP-1 is a major mediator of skin aging, and that inhibition of MMP-1 activity should improve skin health in the elderly.

# 701

**UVA-1 and UVA-1 oxidized phospholipids elicit an autophagic stress response in primary keratinocytes**

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Oxidant stress leads to generation of potentially detrimental modified proteins. Macroautophagy is a candidate mechanism for degradation of damaged cell content, and its role in the stress response of epidermal keratinocytes (KC) is not known. We investigated the role of autophagy after UVA-1 irradiation and UVA-1 oxidized PUFA phospholipids (OxPL). We studied *in vitro* whether exposure to UVA-1 or OxPL, initiate autophagy in primary murine and human KC as measured by immunoblot LC3 conversion assays. We used primary KC of LC3-GFP transgenic marker mice to investigate autophagosome formation. Finally, we investigated the stress responses in KCs from epidermal autophagy deficient mice (ATG7 -/-). Using the LC3 conversion assays, we found that UVA-1 irradiation, treatment with OxPL and the autophagy inducer rapamycin induced LC3 conversion after 4h, indicative of autophagosome initiation. LC3-GFP transgenic KC showed strongly enhanced formation of autophagosomes 6 hours after treatment. The classical marker protein p62 however was not degraded, rather we found high molecular weight complexes positive for p62 to appear upon oxidant stress. Importantly, these complexes were strongly accumulated in samples from stressed autophagy deficient KC. Also baseline protein levels of p62 were elevated in autophagy deficient mice. p62 was described as both, a transcriptional target and an inducer of the Nrf2 antioxidant response (that is activated by UVA-1 and OxPL). Indeed, mRNA and protein levels of Nrf2 dependent genes were significantly elevated in autophagy deficient keratinocytes exposed to oxidant stressors. In conclusion, our data show that autophagy is initiated in KC by UVA-1 and OxPL and may be necessary for the clearance of oxidized/modified protein complexes. Further, loss of autophagy amplifies the Nrf2-antioxidant response in KC, an effect that was reported as detrimental in other epithelia.

# 702

**The combination of 5-fluorouracil and aminolevulinate-photodynamic therapy enhances tumor cell death by targeting p53-MDM2 pathway in murine squamous cell carcinoma tumors**

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Photodynamic therapy (PDT) using 5-Aminolevulinate (ALA) is a well-accepted therapy for skin cancers and precancers. With ALA-PDT, the ALA is selectively retained in tumors to form protoporphyrin IX (PpIX), a photosensitizer that is activated by visible light to induce cell death. ALA-PDT by itself is suboptimal for the treatment of deep tumors. We are interested in ways to improve its effectiveness by increasing the PpIX levels achieved within tumors. Previously we showed that PpIX levels, and tumor cell death, can be significantly enhanced by a combination approach in which Vitamin D or methotrexate is given as a pretreatment prior to PDT. Here, we report that preconditioning of murine tumors with 5-fluorouracil (5-FU) enhances PpIX levels and apoptotic responses after PDT. Experiments were done in two mouse models of skin carcinoma: (1) chemical carcinogen (DMBA-TPA) induced murine tumors; and (2) an SCC model in which human A431 cells were implanted subcutaneously in nude mice. 5-FU was given for 3 days, followed by ALA for 4 h. 5-FU pretreatment caused selective elevations in PpIX levels and tumor death after PDT. 5-FU also stimulated cellular differentiation (E-cadherin), while inhibiting proliferation (Ki67). Because 5-FU is known to cause damage-related upregulation of p53 due to misincorporation into RNA and DNA, we examined levels of p53 and related molecules in the tumors by immunostaining. After 5-FU preconditioning, p53 was strongly upregulated, as were the downstream target molecules p21 and p27. Levels of MDM2, a negative regulator of p53, were reduced. Levels of p19/Arf, which regulates MDM2 stability, were not altered. These results suggest that 5-FU prior to ALA-PDT can improve the therapeutic response of the tumors via enhanced PpIX levels, and tumor cell killing via a mechanism that targets the p53-MDM2 pathway.

# 703

**Semi-targeted tandem mass spectrometry-based lipidomic analysis of oxidized phosphatidylcholines induced by UVA irradiation in dermal fibroblasts**

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Oxidized phospholipids (OxPLs) are increasingly recognized as molecules that are not only markers of oxidative stress but also mediators involved in pathogenesis or resolution of disease states. Different molecular species of OxPLs often have opposing biological activities. UVA-generated non-fragmented OxPCs induce adaptive responses in dermal fibroblasts (FB), while production of fragmented species containing reactive carbonyl groups is correlated to skin ageing and pathologies of the skin that develop upon solar exposure such as actinic elastosis. We report a procedure for analysis of a broad spectrum of molecular species generated by UVA-1 oxidation (40 and 80 J/cm2) of abundant phosphatidylcholines in dermal fibroblasts. The approach is based on two subsequent liquid-liquid extraction steps (acidic methanol/hexane extraction and Folch phase separation) followed by reverse-phase core-shell HPLC coupled to ESI-MS/MS. More than 500 peaks corresponding in retention properties to polar and oxidized PCs were detected within 8 minutes at 99 m/z values in extracts from human dermal fibroblasts. Importantly, 217 of these peaks were fluence-dependently increased upon exposure of cells to UVA irradiation. We confirmed our previous findings that specific biologically active OxPCs are increased by UVA-1 and in addition, we observed accumulation of a plethora of uncharted OxPCs. This opens the possibility to identify novel OxPLs that may either have detrimental effects in photoageing and UV-induced pathologies, but also photoproducts that mediate beneficial effects of UVA radiation, which is utilized for therapy of inflammatory skin diseases. This method may allow characterizing specific "signatures" of OxPCs and to select promising peaks for identification of their molecular structure and biological role.

# 704

**Induction of microRNA miR-21 by UV irradiation down-regulates TGF-β type II receptor and type I procollagen in human skin fibroblasts**

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TGF-β pathway plays a central role in controlling extracellular matrix production by inducing the synthesis and secretion of type I collagen (COL1). Activation of the TGF-β pathway requires type I and type II TGF-β receptors. Ultraviolet (UV) irradiation represses COL1 production through down-regulation of type II TGF-β receptor (TβRII). Here we report that UV irradiation rapidly induces microRNA-21 (miR-21), which directly represses TβRII expression. Induction of miR-21 was observed within 8 hours and remained elevated for 24 hours post UV exposure in human skin *in vivo*. Maximum induction was 4.6-fold at 24 hours (n=6, $p<0.05$). Similar elevations of miR-21 were observed in cultured human skin fibroblasts. Induction of miR-21 was dose dependent; increasing UV dosage from 10 mJ/cm$^2$ to 40 mJ/cm$^2$, increased miR-21 correspondingly from 2.3 fold to 5.8 fold (n=3, $p<0.05$), 6 hours post UV irradiation. Transfection of miR-21 mimic into cultured skin fibroblasts, reduced TβRII mRNA and protein levels by 22% and 62% respectively (n=3, $p<0.05$). Type I TGF-β receptor remained unchanged. In concert with reduction of TβRII, COL1 mRNA and protein were reduced 31% and 46% (n=3, $p<0.05$), respectively. In contrast, transfection of cultured fibroblasts with antisense miR-21 inhibitor increased basal TβRII protein levels 3.2- fold (n=3, $p<0.05$). *In silico* analysis identified potential miR-21 binding sites within the 3′ untranslated region of TβRII mRNA. Insertion of this region into pMIR-REPORT luciferase reporter construct reduced luciferase expression by 32% (n=4, $p<0.05$). Exposure of fibroblasts to UV irradiation further inhibited reporter activity 31% (n=4, $p<0.05$). Repression of reporter activity was abrogated by introduction of antisense miR-21 inhibitor. These data demonstrate that repression of TβRII and COL1 by UV irradiation involves induction of miR-21, which directly binds to specific 3′ untranslated sequences within TβRII mRNA in human dermal fibroblasts.

# 705

**Matrix metalloproteinase expression and nano-scale quantitative surface morphometric analysis of photodamaged human dermis**

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Solar ultraviolet (UV) irradiation induces matrix metalloproteinases (MMPs), which degrade collagen fibrils and other components of the dermal extracellular matrix (ECM). This ECM degradation accumulates with chronic sun exposure, leading to permanent structural and functional impairment, which deleteriously impacts the health and appearance of skin. We quantified gene expression of all 23 members of the MMP family in clinically-photodamaged forearm and matched sun-protected underarm skin, from 19 individuals. Multiple MMPs were constitutively elevated in photodamaged forearm skin (fold elevation from high to low: MMP-9, 5.3-fold; MMP-27, 5.1-fold; MMP-13, 3.8-fold; MMP-3, 3.0-fold; MMP-11, 2.7-fold; MMP-17, 2.2-fold; MMP-1, 1.9-fold; MMP-2, 1.6-fold; MMP-15, 1.3-fold, all $p<0.05$). To determine the relative contribution of epidermis and dermis to MMPs elevations in photodamaged skin, epidermis and dermis were separated by laser capture microdissection. All MMPs, except MMP-3, were primarily expressed in the dermis. (MMP-27, 86%; MMP-9, 85%; MMP-2, 83%; MMP-11, 82%; MMP-15, 73%; MMP-17, 62%; MMP-1, 59%; MMP-13, 52%). Atomic force microscopy (AFM) nanoscale-images revealed that collagen fibrils in sun-exposed forearm dermis were fragmented, sparse, and disorganized. Quantitative analysis of AFM data indicated that the average roughness (a measure of fibril organization) of dermal collagen fibrils was five-times greater in photodamaged forearm, compared to sun-protected underarm dermis (59.1nm vs. 12.3nm, $p<0.01$). Our data indicate that compared to acute UV irradiation, in which only three MMPs (MMP-1, 3, and 9) are transiently induced primarily in epidermis, photodamaged skin constitutively expresses elevated levels of additional MMPs in the dermis. These elevated multiple MMPs likely lead to chronic, progressive degradation of dermal ECM, which contributes to connective tissue in photodamaged human skin.

# 706

**IL-11, IL-1α, IL-6, and TNFα are induced by solar radiation and involved in the facial subcutaneous fat loss**

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Interleukin-11 (IL-11) is known as an adipogenesis inhibitory factor which hinders preadipocyte differentiation *in vitro* and is induced by cytokines (i.e. IL-1, TGF-β) in fibroblasts. Previously we showed that IL-11 can be induced by solar irradiation in dermal fibroblasts to consequently inhibit preadipocyte differentiation and that sunscreens prevented the solar-induced IL-11 production and subsequent inhibition of preadipocyte differentiation. Here, we demonstrate that in addition to IL-11, other cytokines such as IL-1α, IL-6 and TNFα are expressed and secreted from solar-irradiated skin equivalents. Sunscreens significantly inhibited the release of these cytokines. Facial preadipocytes were treated with media from skin equivalents that were exposed to a solar simulator in the absence and presence of neutralizing antibodies against these cytokines. UV exposure was found to inhibit preadipocyte differentiation. The inhibition of preadipocyte differentiation was prevented when the medium were pre-treated with a cocktail mixture of neutralizing antibodies to IL-11, IL-1α, IL-6, and TNFα as compared to that of the non-treated or IgG-pre-treated conditioned media. These results suggest that a) the solar irradiation-induced release of IL-11, IL-1α, IL-6, and TNFα is involved in the facial subcutaneous fat loss and b) sunscreens may prevent the UV-induced changes in facial contouring by blocking the release of these pro-inflammatory cytokines.

# 707

**Cyclophilin D alters UVA-induced apoptosis in keratinocytes through a mechanism involving mitochondrial ROS generation**

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Cyclophilin D (CyPD) is a mitochondrial target of CsA playing an integral role in mitochondrial permeability transition pore formation and consequently in cell death processes. In this study, we modeled two keratinocyte cell lines with CyPD being stably knocked down using viral particles containing shRNA for CyPD. The expression level of CyPD transcripts confirmed by qPCR was knocked down by 90 to 99%. Proliferation rate of the cells with silenced CyPD was largely decreased with a significantly higher apoptotic rate in these cells compared to controls. However, after UVA irradiation the apoptosis was found to be vastly lower in CyPD silenced cells than it was in control cells where the UVA treatment resulted in significantly higher apoptosis. Moreover, mitochondrial membrane potential was found to be less dissipated and mitochondrial transition pore less active in cells with knocked down CyPD than in control cells after UVA irradiation. Furthermore, less superoxide was detected in the cells with silenced CyPD compared to controls after UVA exposure but more superoxide was found in CyPD silenced cells without UVA irradiation. Cells with knocked down CyPD are slightly more apoptotic under baseline conditions while being more resistant to UVA-induced apoptosis. The ability of CyPD to regulate UV induced apoptosis may indicate a role of CyPD as a potential target in cancer therapy. Our data show that diminished levels of CyPD expression are associated with increased resistance to UVA-induced apoptosis. Therefore, in skin, therapeutic strategies to lower levels of CyPD may have an adverse effect on tumors in that they may be in fact more resistant to specific apoptotic stimuli such as UV light. Moreover, the effect could be counterproductive leading to the cancer progression. As a corollary, therapeutic strategies to increase CyPD levels in cutaneous malignancies may allow for UVA to be used as a paired therapeutic modality to treat skin cancers with depths that are penetrable by UVA irradiation.

# 708

**Upregulation of Srcasm in aged, sun-damaged skin**

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Src kinases are key regulators of keratinocyte growth and differentiation. Srcasm is a negative regulator of Src kinases, and low Srcasm levels are associated with keratinocyte proliferation while high Srcasm levels are associated with keratinocyte differentiation and senescence. We hypothesized that aged skin demonstrates decreased Src kinase activity and increased Srcasm levels. To address the hypothesis, the levels of Src tyrosine kinase activity and Srcasm were determined by immunohistochemistry in the following: 1) Sun-exposed skin (forearm) from humans greater than or equal to the age of 70 (N = 10), 2. Sun-exposed skin (forearm) from humans less than or equal to the age of 30 (N = 10), 3. Sun-protected skin (buttock) from humans greater than or equal to the age of 70. (N = 10), 4. Sun-protected skin (buttock) from humans less that or equal to the age of 30. (N = 10). Immunohistochemistry was performed to detect phospho-Src (p-Src), phospho-EGF receptor (p-EGFR), Srcasm, Ki-67, involucrin, and b1-integrin. Statistically significant differences in Srcasm and Ki-67 levels between young skin and aged skin were shown. Using a semi-quantitative scale, the staining index of Srcasm was significantly higher in aged, sun-damaged skin compared to young sun-protected, young sun-exposed, and aged sun-protected skin ($p = 0.0094$). The Ki-67 staining index for sun-exposed aged skin was inversely related to Srcasm levels. In sun-damaged aged skin, Srcasm staining was noted in the basal layer of the epidermis which was not seen in the other sample groups. These data suggest an inverse relationship between Ki-67 and Srcasm in sun-damaged aged skin. Our data suggest that chronic sun exposure in aged skin is associated with increased Srcasm in the basal cell layer and decreased Ki-67 staining; the impaired keratinocyte proliferative response in aged sun-damged skin may be secondary to elevated Srcasm levels.

# 709

**Advances in the protection and reparation of epidermal cells from UV-induced DNA damages**

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Acute exposure to UV radiations results in erythema, immune suppression and cell death, while chronic exposure results in photoaging and carcinogenesis. α-Melanocyte-stimulating hormone (α-MSH) induces epidermal pigmentation upon binding to the melanocortin-1 receptor, which in turn activates the signal transduction pathway leading to the induction of tyrosinase and other key players for melanin synthesis. In addition, it has been proven that α-MSH directly reduces UV-induced DNA damage and enhances DNA repair in epidermal melanocytes and keratinocytes, by modulating the function of DNA repair molecules. The objective of this study was to investigate whether a biomimetic peptide analogue of α-MSH, acetyl hexapeptide-1, displays protective effects *in vitro* against UV genotoxicity and UV-induced DNA damages in human epidermal cells and *ex vivo* skin, as well as protective properties against sun-induced erythema, after a treatment of 14 days of 27 human volunteers. We demonstrated that acetyl hexapeptide-1, reduces the genotoxic adverse effects of both UVA and UVB in melanocytes, and that it protects keratinocytes from DNA oxidative damages induced by UVA. Furthermore, using an *ex vivo* skin model, we showed that acetyl hexapeptide-1 significantly reduces the appearance of CPDs after UV challenge, and that it blocks UV-induced caspase-3 activation and consequently apoptosis. Clinically this biomimetic peptide demonstrated its anti-erythemal properties after 14 days preventive topical skin application showing a significant increase (+20%) of the Minimal Erythemal Dose compared to placebo. In conclusion, we demonstrated a global approach on preventive and protective effects of acetyl-hexapeptide-1 against UV-induced damages to skin cells. Our demonstrations include several specific classical tests but also a new biological pathway through caspase-3 inhibition, a protease implicated in the apoptosis convergence point pathway.

# 710

**The influence of extracorporeal photochemotherapy on global gene expression profiles in lymphocyte subsets**

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Extracorporeal photochemotherapy (ECP) is a treatment for cutaneous T cell lymphomas, graft-versus-host disease (GvHD), organ rejection, systemic sclerosis, and other immune-mediated disorders. During ECP peripheral blood leukocytes are exposed to 8-MOP and UVA prior to reinfusion to the patient. Only limited information is available on mechanisms of action of ECP and its influence on gene expression in treated cells is currently unknown. In the present study we used Affymetrix® Human Genome U133 Plus 2.0 Arrays to compare global gene expression profiles in CD4+ and CD8+ lymphocytes before and after ECP. 6 female patients with chronic GvHD were included in the study. CD4+ and CD8+ lymphocyte subsets were isolated before and immediately after exposure to 8-MOP/UVA during a standard ECP treatment cycle. Total RNA was isolated from each cell sample and processed and analyzed according to standard procedures. We found that exposure to 8-MOP/UVA during ECP induced an immediate and specific molecular response in CD4+ and CD8+ lymphocytes in patients with GvHD. There was a striking difference between CD4+ and CD8+ subsets in numbers of regulated genes. In CD4+ cells 101 genes showed significant regulation (48 up, 53 down; false recovery rate adjusted $p<0.01$). Applying the same criteria in CD8+ cells only 6 downregulated genes could be detected. These results provide for the first time a database of ECP regulated genes which is not based on a preformed hypothesis. Thus it provides an unbiased look on molecular events that might underlie the therapeutic efficacy of ECP. Confirmation and further functional analysis of genes detected in this study as well as extension of these experiments to other leukocyte subsets and other diseases will help to define targets for further ECP-related research.

# 711

**The role of α-melanoctye stimulating hormone in the DNA damage response of human melanocytes**

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We have reported that α-melanocyte stimulating hormone (α-MSH) reduces DNA damage by enhancing nucleotide excision repair and reducing oxidative DNA damage via activating the melanocortin 1 receptor (MC1R). Treatment of human melanocytes (MC) with 10nM α-MSH for 4 days followed by irradiation with 75 mJ/cm2 significantly increased the levels of the DNA damage sensors ATR and ATM in the α-MSH+UV group compared to the UV group at 45 min and 2 hrs post UV. These sensors activate a cascade of events including phosphorylation of H2AX (γH2AX), which is important for the maintenance of genomic stability. α-MSH increased γH2AX in a UV dose- (25-105 mJ/cm2) and time-dependent manner (peaking at 6-8 hrs post UV), as measured using flow cytometry, immunocytochemisty and Western blotting. This elevation in γH2AX occurred in all phases of the cell cycle. Similar effects on γH2AX were observed with forskolin, suggesting that activation of the cAMP pathway increases H2AX phosphorylation. No change in γH2AX following α-MSH treatment was noted in MC with loss of function MC1R, which increases melanoma risk. Pretreatment of MC with the antioxidant N-acetyl cysteine had no effect on the α-MSH induced increase in γH2AX, suggesting that γH2AX is primarily induced by DNA photoproducts and DNA repair intermediates. Caffeine, a general inhibitor of ATR, ATM and DNA-PK, and KU55933, a specific ATM inhibitor, significantly reduced the α-MSH induced increase in γH2AX. Further experiments showed the activation of the downstream effector kinases, checkpoint kinases 1 and 2 in the UV and α-MSH+UV groups beginning as early as 1hr post UV. The p53 dependent phosphatase WIP1 known to dephosphorylate γH2AX was increased in the α-MSH+UV treated MC compared to the UV only group at 24 hrs post UV irradiation, which is coincident with significant reduction in γH2AX. These results demonstrate that α-MSH plays an important role in regulating the DNA damage response and underscores the importance of the MC1R in the maintenance of genomic stability in MC.

# 712

**Longwave ultraviolet light (UVA) accelerates intrinsic aging by inducing progerin and by impairing shape and function of nuclear membranes**

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Premature aging of many organs in Hutchinson Gilford progeria syndrome (HGPS) is caused by a mutation of the LMNA gene that activates a cryptic splice site. This results in expression of a truncated form of Lamin A, called progerin. Accumulation of progerin in the nuclei of HGPS cells impairs nuclear functions and causes abnormal nuclear morphology with blebbing and invaginations of the nuclear membrane. Recently, accumulation of progerin has also been described during normal intrinsic aging. We hypothesized that accumulation of progerin and progerin-mediated dysfunction of the nuclear membrane (HGPS-like phenotype) may also characterize photoaging (extrinsic aging). We therefore exposed cultured fibroblasts from neonates and aged donors, as well as fibroblasts aged in culture to single or repeated doses of UVA or UVB and assessed cells for induction of a HGPS-like phenotype. Repeated exposures of growth-arrested cells to UVA (5 J/cm$^2$, 5 times a week for 3 weeks) induced progerin, as detected by Western blotting and immunocytochemistry. Progerin was not detected in sham- or UVB-irradiated cells. Real-time quantitative PCR revealed a prominent, 5.1-fold induction of progerin mRNA 24 hrs after a single exposure to UVA (10 J/cm$^2$), and only a less prominent, 1.9-fold induction after UVB (30 mJ/cm$^2$). Staining the nuclear membranes of repeatedly irradiated cells for Lamin A showed a dose-dependent increase of abnormal, HGPS-like nuclear shapes after UVA exposures, more than after UVB exposures (e.g. severely abnormal nuclei in 15.9 and 28.9 % of cells irradiated with 2 or 5 J/cm$^2$ UVA, respectively, as compared to 4.9 % and 12.4 % in sham- or UVB-irradiated cells). These data indicate that an HGPS-like cellular phenotype not only occurs during intrinsic aging, but is also induced by UV, in particular by longwave UVA. This suggests that at least some aspects of photoaging should be regarded as a process of damage-accelerated intrinsic aging.

# 713

**Immunohistochemical characterization of chronic ultraviolet damage in human skin**

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In this IRB-approved study, we compared the immunohistochemical staining patterns and intensity of markers of oxidative stress in matched chronically sun-exposed and sun-protected skin biopsies. Compared to sun-protected skin, the staining intensity of 4-hydroxy-2-nonenal (HNE), 8-hydroxy-deguanosine (8-OH-dG) and advanced glycation end products (AGEs) in chronically sun-exposed skin biopsies is significantly increased. The staining intensity of HNE in sun-exposed skin samples was 185% ± 0.22 (standard error of the mean (SEM)) ($p < 0.001$) for stratum corneum, 158% ± 0.18 ($p < 0.05$) for epidermis and 163% ± 0.23 ($p < 0.67$) for dermis as compared to sun-protected skin samples. The staining intensity of 8-OH-dG in the nuclei of sun-exposed skin samples was determined to be 202% ± 0.29 ($p < 0.001$) for epidermis and 123% ± 0.29 ($p < 0.05$) for dermis as compared to sun-protected skin samples. The staining intensity of AGEs in sun-exposed skin samples was 191% ± 0.17 ($p < 0.05$) for stratum corneum, 196% ± 0.16 ($p < 0.001$) for epidermis and 193% ± 0.19 ($p < 0.001$) for dermis as compared to sun-protected skin samples. The staining patterns of HNE, 8-OH-dG and AGEs in sun-exposed skin compared to sun-protected skin represent the effect of chronic oxidative damage secondary to ultraviolet (UV) radiation that occurs in all layers of the skin. These findings aid in the understanding of the role of oxidative damage in photoaging. The use of these three immunostains together can be used as a panel for future evaluation of products, as they become available, that prevent oxidative damage to the skin.

## 714

**A topical NF-κB inhibitor protects skin cells from photodamage and enhances DNA repair**

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In addition to the natural aging process, our skin is under continuous assault from a variety of damaging environmental factors including solar ultraviolet (UV) radiation and atmospheric pollutants. The cumulative damage to our skin from these environmental aggressors increases with age, and can result in oxidative stress, chronic inflammation and even DNA damage. The transcription factor NF-κB is a key regulator of inflammatory mediators, cell proliferation and apoptosis in skin cells. This pathway can be activated in response to ultraviolet radiation leading to the production of pro-inflammatory cytokines and matrix metalloproteases (MMPs) causing photo damage to skin. The effects of a topical NF-κB inhibitor were evaluated in human epidermal skin equivalents exposed to UV radiation. The NF-κB inhibitor successfully mitigated UV-induced pro-inflammatory cytokines, reactive oxygen species and MMPs, which can lead to degradation of the extracellular matrix. Furthermore, topical application of this inhibitor also suppressed markers of UV-induced DNA damage such as T-T dimers. In a comet assay, pretreatment with the NF-κB inhibitor enhanced the repair of DNA damage caused by UV in primary keratinocytes. Similar results were also observed using BAY 11-7082, a specific and potent, inhibitor of NF-κB. Taken together, these results demonstrate that, topical application of a NF-κB inhibitor mitigates skin inflammation and UV-induced photo damage, in addition to inducing DNA repair in skin cells.

## 715

**Photoprotection against UV damage by a stabilized silicone gel matrix antioxidant formulation**

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UV radiation is a potent generator of oxidative stress and a major cause of skin photodamage leading to premature skin aging and skin cancer. UV radiation causes DNA damage as evidenced by an increase in thymine dimers and sunburn cells. This study describes the photoprotective effects of a uniquely formulated, stabilized silicone gel matrix antioxidant formulation containing Vitamin C and E. For the *in vitro* studies, Epiderm tissues (were treated topically with test material, positive control) or negative control and then exposed to UVB (300 mJ/cm2). DNA was extracted from Epiderm tissues and assayed for thymine dimer content. The test material showed a 70% reduction in thymine dimer content in tissues 24 hours post UVB exposure compared to untreated control ($p<0.05$) which was confirmed by immunohistochemical staining . Examination of sunburn cells in H&E stained tissues showed significant protection against sunburn cell formation when treated with in test material. To evaluate its ability to prevent UV mediated inflammation and pigmentation *in vivo*,17 healthy female subjects, aged 26-63 years with Fitzpatrick Skin Types III-IV completed a single-center, double-blinded comparison study. Test products were applied once daily for 4 days onto the backs of the subjects. The sites were then irradiated with a solar simulator. Application of test products resumed on Day 6-20. Standardized digital photographs were taken on Days 6 and 20. The images were analyzed using a computer-aided colorimetry algorithm, to determine a* (degree of redness) and L* (brightness). At day 6, Vit C&E product showed a statistically significant change in a* ($P=0.01$) and L* ($P<0.001$) when compared to untreated control, demonstrating prevention of initial UV mediated damage. At Day 20, Vit C&E product continued to show significantly higher L* compared to untreated control ($P<0.001$) demonstrating protection against post-inflammatory hyperpigmentation. These studies demonstrate that substantial photoprotection can be achieved by this unique antioxidant formulation.